Local and remote lesions in horses subjected to small colon distension and decompression

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Abstract

The purpose of this study was to observe and characterize colonic and lung lesions in horses subjected to experimental distension and decompression of the small colon. Sixteen healthy adult horses were divided into 2 groups: 9 horses that were subjected to distension of the small colon by means of a latex balloon surgically implanted in the lumen and inflated to a pressure of 40 mm Hg for 4 h, and 7 horses in which the balloon was implanted but not inflated. Colonic biopsy specimens were collected before balloon implantation, at the end of the period of obstruction, and 1.5 and 12 h after decompression and were examined for hemorrhage, edema, and neutrophil infiltration; myeloperoxidase (MPO) activity and hemoglobin concentration were measured as well. At the end of the experiment, lung samples were also collected and examined for neutrophil accumulation and MPO activity. The mucosa was not affected by luminal distension; lesions were restricted to the seromuscular layer. Neutrophil accumulation and edema were observed in the samples from both groups of horses but were greater in those from the distension group, in which there was also hemorrhage, fibrin deposition, and increased MPO activity in the seromuscular layer. Similarly, there was greater accumulation of neutrophils in the lung samples from the distension group than in those from the sham-operated group, as determined by histologic evaluation and MPO assay. These findings provide new evidence of reperfusion injury and a systemic inflammatory response, followed by remote lesions, in horses with intestinal obstruction.

Résumé

Le but de cette étude était d'observer et de caractériser les lésions au côlon et au poumon chez des chevaux soumis à une distension et décompression expérimentale du petit côlon. Seize chevaux adultes ont été divisés en 2 groupes : 9 chevaux qui ont été soumis à une distension du petit côlon au moyen d'un ballon de latex implanté chirurgicalement dans la lumière et gonflé à une pression de 40 mm Hg pour 4 h, et 7 chevaux chez qui le ballon a été implanté mais non gonflé. Des spécimens de biopsie du côlon ont été prélevés avant l'implantation du ballon, à la fin de la période d'obstruction et 1,5 et 12 h après décompression et ont été observés pour la présence d'hémorragie, œdème, et infiltration de neutrophiles; l'activité de la myéloperoxidase (MPO) et la concentration d'hémoglobine ont également été mesurées. À la fin de l'expérience, des échantillons de poumon ont été prélevés et examinés pour l'accumulation de neutrophiles et l'activité de la MPO. La muqueuse n'était pas affectée par la distension de la lumière intestinale; les lésions étaient limitées à la couche séromusculaire. L'accumulation de neutrophiles et l'œdème étaient observés dans les échantillons provenant des deux groupes de chevaux mais étaient plus marqués chez les chevaux soumis à une distension, chez qui on observa également des hémorragies, la déposition de fibrine et une activité de la MPO augmentée dans la couche séromusculaire. De manière similaire, il y avait une plus grande accumulation de neutrophiles dans les échantillons de poumon provenant des chevaux du groupe ayant subit une distension que chez les chevaux du groupe témoin, tel que déterminé par l'évaluation histologique et les résultats des épreuves de la MPO. Ces résultats fournissent de nouvelles évidences des dommages de reperfusion et de réponse inflammatoire systémique, suivi de lésions à distance, chez des chevaux avec obstruction intestinale.

(Traduit par Docteur Serge Messier)

Introduction

In the last 2 decades, several experimental models of intestinal ischemic obstruction in horses have been developed. One of the most interesting facts revealed by such models is that lesions can progress even after restoration of blood flow to the ischemic segment. In some studies, lesions caused by reperfusion after ischemia were even greater than those observed after the same period of uninterrupted ischemia (1,2). This demonstrates that the reperfusion phase can be as important as the ischemic phase, or more so, and indicates that it can contribute to postoperative complications and worsen the prognosis.

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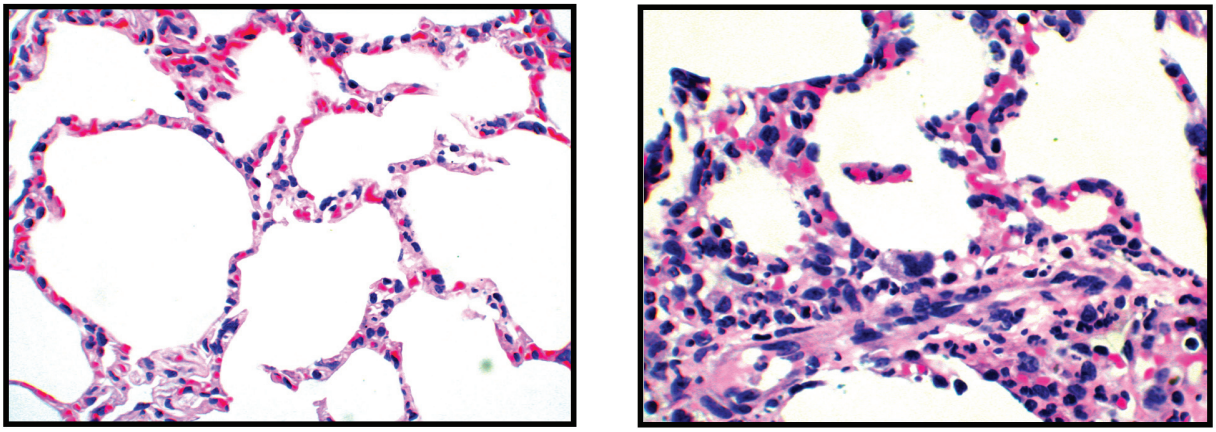

Figure 1. Lung sections from horses in the sham-operated group (left) and the distension group (right), showing few neutrophils in the former and many in the latter at the end of the experiment. Hematoxylin–eosin; original magnification ×400.

Reperfusion injury has been extensively studied in laboratory animals. The effects are mainly related to the generation of oxygen free radicals, increases in the intracellular concentration of calcium, and neutrophil accumulation (3). Besides the local effects in the ischemic segment, lesions in other parts of the affected organ and even in distant organs, especially the lungs, have been described after intestinal ischemia and reperfusion (3). In horses, limited evidence of neutrophil accumulation in pulmonary vessels of ponies (4) and increased pulmonary artery pressure (5) were reported after experimental large colon ischemia, but otherwise there have not been descriptions of remote lesions in the lungs.

Intestinal ischemia in horses is easily recognized in cases of strangulating obstruction; however, there is increasing evidence that ischemia can be important in intestinal distension due to mural compression (6,7). Intestinal distension has also been associated with poor prognostic indicators and with postoperative complications such as adhesions (8) and paralytic ileus (9).

Recently it was shown that distension of the equine small colon with a luminal pressure of 40 mm Hg decreased mural perfusion to 26.4% of that of control segments (7). This model, when used to produce small colon obstruction for 3 h, induced significant alterations in the peritoneal fluid, a potential indicator of intestinal injury (10).

We hypothesized that luminal distension of the equine small colon for 4 h would cause ischemia–reperfusion lesions both locally and in other parts of the small colon and that the lesions would be more evident in the seromuscular layer. Additionally, we would expect to find evidence of damage in the lungs. The objectives of the study reported here were to use a model of small colon luminal distension to characterize local and remote lesions at the end of 4 h of distension and at 1.5 and 12 h after decompression and to search for lesions in other areas of the small colon and the lungs.

Materials and methods

Animals

The experimental protocol was approved by the Committee of Ethics and Animal Welfare of the Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista, Jaboticabal, Brazil, and was performed under international guidelines for the care and use of experimental animals. The 16 clinically normal horses (5 geldings and 11 nonpregnant mares) had a mean age (and standard deviation) of 3.1 (2) y, mean weight of 351 (38) kg, and a body condition score of 3 to 4 out of 5. The horses were treated with ivermectin (200 μg/kg, given orally) and were maintained for 15 d on a standard diet of hay and commercial equine food before the study.

The horses were divided into 2 groups: 9 horses were subjected to distension of the small colon by means of a latex balloon surgically implanted in the lumen and inflated to a pressure of 40 mm Hg for 4 h; for the other 7 horses, the balloon was implanted but not inflated.

Instrumentation

After 12 h of withholding of feed but not water, the horses were restrained in stocks and sedated with xylazine (0.5 mg/kg given intravenously [IV]) and butorphanol (0.1 mg/kg given intramuscularly [IM]). Local anesthesia of the left flank was achieved with an inverted "L" technique and a combination (1:1) of 2% lidocaine and 0.5% bupivacaine. Through left-flank laparotomy, the oral one-third of the small colon was exposed and exteriorized. A 5-cm-long enterotomy was performed along the antimesenteric surface approximately 50 cm from the transition of the transverse colon and immediately after the intestinal segment supplied by the 1st radial branch of the mesenteric artery. A 15-cm-diameter latex balloon with an extension tube attached to an inflating pump was inserted orad to the incision. To avoid displacement of the balloon in either an oral or aboral direction, the small colon on either side was loosely clamped with circumferential Penrose tubing applied in a manner that avoided occluding mesenteric and mural perfusion. The tubing was fixed on the antimesenteric border with 0 monofilament nylon in a Sultan suture pattern. The enterotomy was performed beyond the site where the Penrose tubing was located and was closed with 2-0 polyglactin 910 in a simple continuous suture pattern, with the extension tube exiting at the edge of the wound; the small colon was repositioned in the peritoneal cavity.

In the distension group, the balloon was inflated to a pressure of 40 mm Hg, which has been shown to decrease mural perfusion to

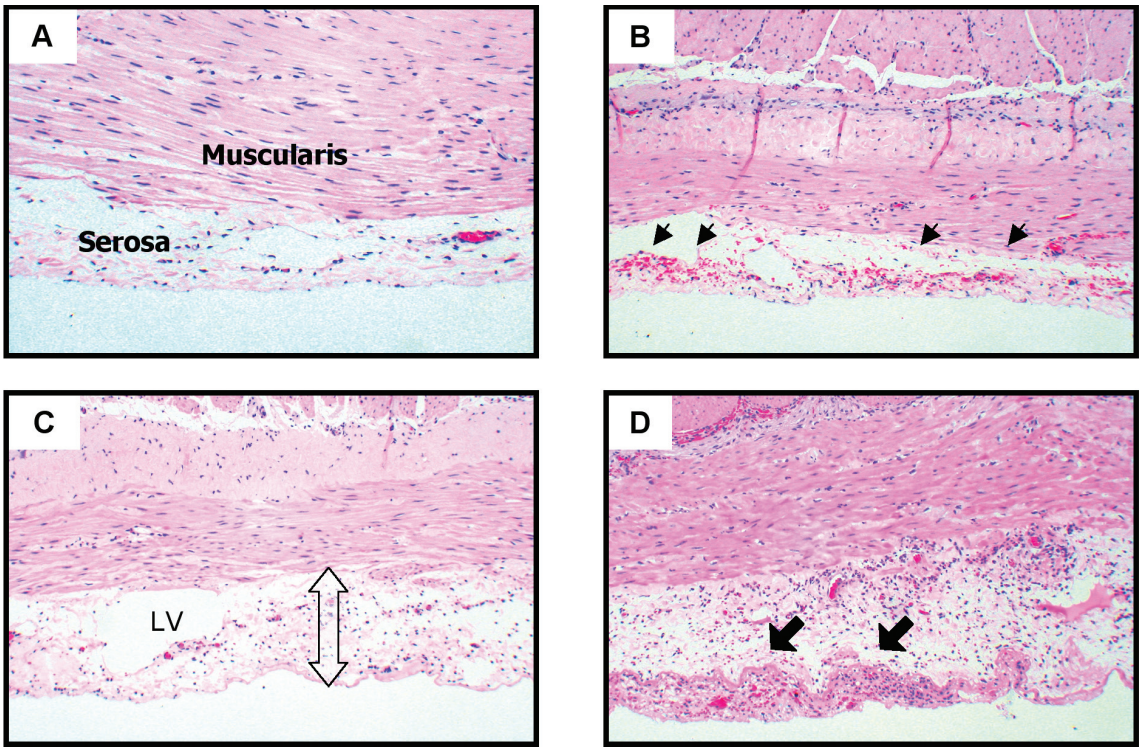

Figure 2. Seromuscular layer of equine small colon before intraluminal distension (A), during (B), and afterwards (C,D), demonstrating (B) hemorrhage (thin arrows) after 4 h of distension, (C) inflammatory cell infiltration, serosal edema (open arrow), and lymphatic vessel (LV) distension by 1.5 h after decompression, and (D) intense tissue infiltration of inflammatory cells and erythrocytes and fibrin deposition over the serosa (thick arrows) at 12 h after decompression. Hematoxylin–eosin; original magnification ×100.

26.4% of that in intact segments (7). In the sham-operated group, the balloon was filled with air but not enough to cause positive pressure inside the balloon or distension of the intestinal lumen. In both groups, the abdominal wall was closed with polyamide (0.60 mm) in a simple continuous suture pattern, the extension tube exiting at the edge of the incision.

The pressure inside the balloon was recorded by a previously calibrated polygraph (7-8P-24.5; Grass Instrument Division, Astro-Med, West Warwick, Rhode Island, USA) connected to the extension tube by a pressure transducer (P23XL; Ohmeda, Madison, Wisconsin, USA). In the distension group, the luminal pressure was checked for 10 min and then every 30 min, and air was added or removed from the balloon when necessary to maintain the pressure as close as possible to 40 mm Hg.

After that, the horses were premedicated with midazolam (0.15 mg/kg IV) and guaifenesin (100 mg/kg IV), and anesthesia was induced with thiopental (3 mg/kg IV). The animals were positioned in dorsal recumbency, and anesthesia was maintained with halothane in oxygen. The mean arterial pressure (MAP), measured by facial artery catheterization, was kept between 70 and 100 mm Hg; the carbon dioxide pressure (P_{CO_2}) was kept at 55 mm Hg or less. Through a 30-cm midline celiotomy, the small colon was exposed. After 4 h of distension, the balloon was deflated and removed, the enterotomy site closed, and the experimental segment returned to the abdominal cavity. After 90 min, the abdominal wall was closed by routine techniques, and the horse was allowed to recovery from anesthesia.

At the beginning of the recovery period, the horses were treated with buprenorphine (0.005 mg/kg IM) to provide analgesia. Twelve hours after the balloon was removed, they were sedated with xylazine (1 mg/kg IV) and acepromazine (0.1 mg/kg IV), anesthesia was induced with thiopental (15 mg/kg IV), and they were euthanized with an infusion of a saturated solution of magnesium sulfate given IV.

Sample collection and preparation

Full-thickness samples (2 × 5 cm) were collected from the antimesenteric surface of the small colon parallel to the taenia at the border of the experimental segment before balloon placement, at the end of the obstruction period, and at 1.5 and 12 h after removal of the balloon. The biopsy sites were sutured with a simple continuous pattern followed by a Cushing's pattern in the seromuscular layer. The small colon was then returned to the abdominal cavity and the midline incision apposed with Backhaus tongs and covered with moistened towels until the next sampling time. At the end of the experiment, similar samples were obtained from the small colon in an aboral segment about 1 m distant to the site of obstruction. At this time, 1 to 3 lung samples approximately 1 cm^3 were taken from various sites.

Intestinal samples were divided into 2 portions. One portion was fixed in 10% neutral buffered formalin and embedded in paraffin; sections 5 μm thick were stained with hematoxylin–eosin. The 2nd portion was placed in aluminum foil and immediately frozen by immersion in liquid nitrogen. The lung fragments were divided

in 2 portions; 1 portion was processed for light microscopy, and the other was frozen as described for the colon samples.

Macroscopic and histopathologic evaluation

At the time of biopsy and necropsy, the small colon was macroscopically examined for alterations in color and wall thickness and for the presence of edema, blood, and fibrin.

By light microscopy, the colon samples were blindly evaluated in a semiquantitative manner for mesothelial cell detachment, serosal fibrin deposition and lymphatic vessel distension, mucosal epithelial cell detachment, and edema, hemorrhage, and neutrophil accumulation in all layers. A scale from 0 to 5 was used for epithelial cell detachment (11), from 0 to 4 for edema, hemorrhage, and neutrophil accumulation (0 — absent; 1 — discrete; 2 — mild; 3 — evident; 4 — intense), and from 0 to 3 for mesothelial cell detachment, fibrin deposition, and lymphatic vessel distension (6). In the lung samples, the number of neutrophils was scored from 0 to 3 (0 — rare; 1 — few; 2 — moderate; 3 — many). Neutrophils were counted in 15 randomly selected fields in each section with the use of an ocular grid and a 100x immersion objective superimposed over the middle of the serosal and external muscular layer.

Biochemical analyses

Neutrophil accumulation in isolated samples of the seromuscular layer of the small colon and lung was measured by assaying myeloperoxidase (MPO) activity as previously described (12). Thawed tissue was homogenized in buffer (0.1 M NaCl, 0.02 M Na_3PO_4, 0.015 M sodium ethylene diamine tetraacetic acid; pH 4.7), 1 g of tissue per 19 mL of buffer, and centrifuged at 260 $\times$ *g* for 10 min. The pellet was subjected to hypotonic lyses (15 mL of 0.2% NaCl solution, then, 30 s later, the addition of an equal volume of a solution of 1.6% NaCl and 5% glucose). After further centrifugation, the pellet was resuspended in 0.05 M Na_3PO_4 buffer (pH 5.4) containing 0.5% hexadecyltrimethylammonium bromide (HTAB) and rehomogenized. Aliquots (1-mL) of the suspension were transferred into 1.5-mL Eppendorf tubes and subjected to 3 freeze–thaw cycles with liquid nitrogen. The aliquots were then centrifuged for 15 min at 10 000 $\times$ *g*. Myeloperoxidase activity in the supernatant was assayed by measuring the change in optical density (OD) at 450 nm with the use of tetramethylbenzidine (1.6 mM) and H_2O_2 (0.5 mM). The results were expressed as optical density (OD) per 100 mg of tissue.

Statistical analyses

Where indicated, data are reported as mean (and standard error). Parametric data underwent analysis of variance followed by the Student–Newman–Keuls post-hoc test. Semiquantitative morphologic data were analyzed with the use of Friedman's test of differences among times in the same group and the Mann–Whitney test for differences among groups at the same time. Statistical significance was set at $P < 0.05$ for all tests.

Results

Lung samples were collected from 7 horses in the distension group, but the samples for 3 horses were removed from statistical analysis because of evidence of previous disease that had not been detected by clinical evaluation. All 3 horses had intense neutrophil accumulation in the parenchyma, but 2 also had bronchiolar exudates, and the samples for 1 horse showed eosinophil infiltration.

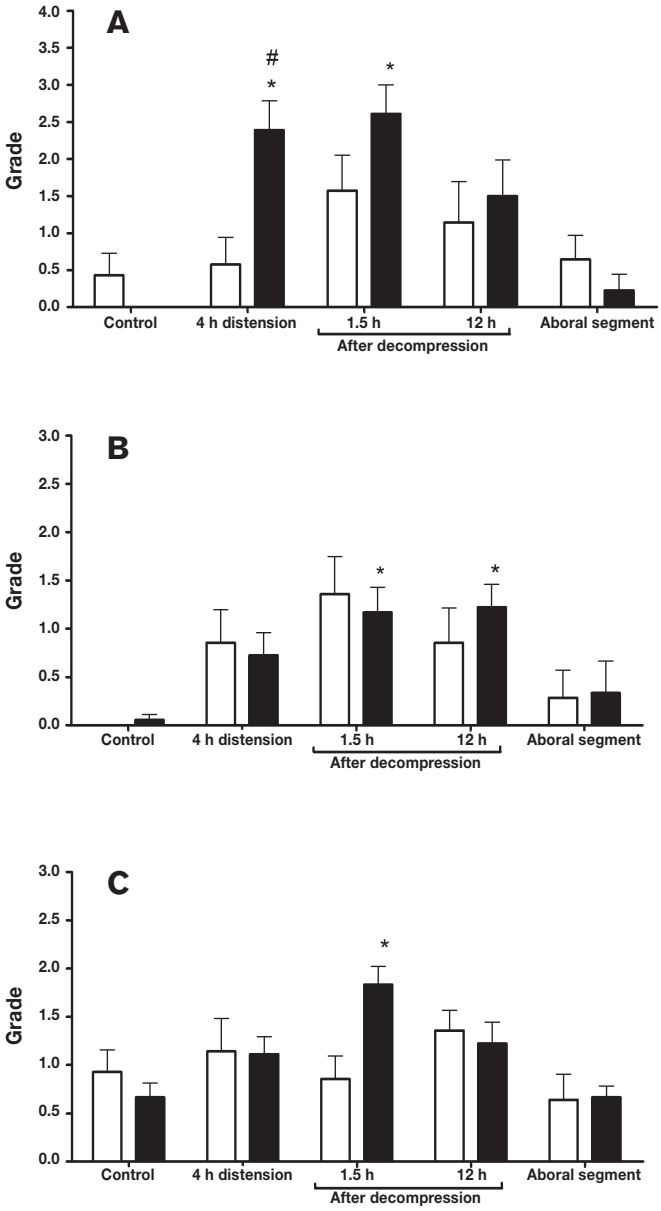

Figure 3. Mean grades (and standard error) for serosal hemorrhage (A), fibrin deposition (B), and lymphatic vessel distension (C) in horses undergoing 4 h of colonic distension and then decompression (*n* = 9; dark bars) or a sham operation (*n* = 7; white bars). In all the figures, the asterisks indicate significant differences from the control segments of colon, and the number signs indicate a significant difference between the 2 groups of horses ($P < 0.05$).

The remaining lung samples from the distension group showed moderate to intense neutrophil accumulation in the microvasculature that apparently did not reach the interstitial space (Figure 1). In the sham-operated group, only 1 horse had obvious intravascular neutrophil accumulation in the lungs.

At the end of the distension period, the experimental segments of colon had a thin wall through which the pale yellow of the balloon

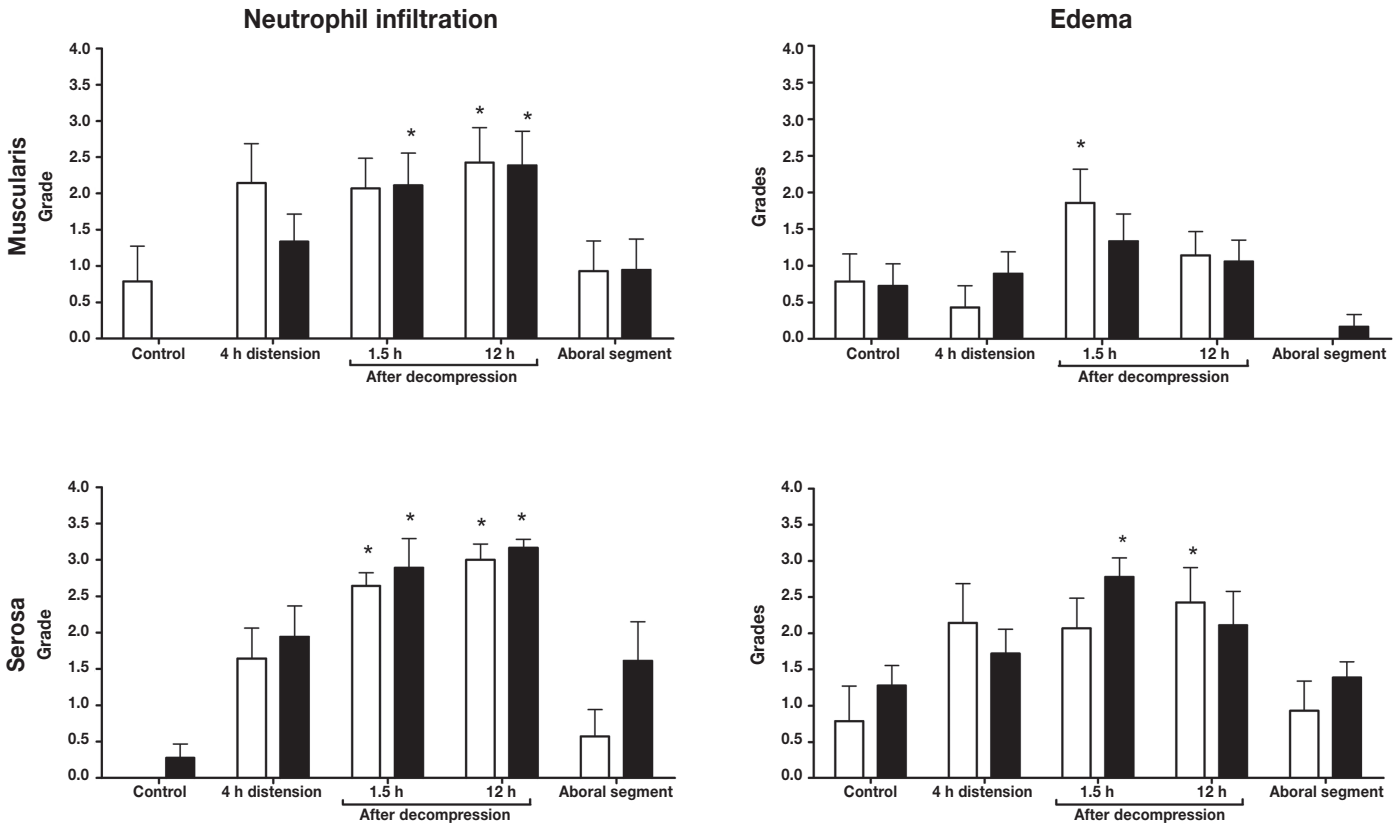

Figure 4. Mean grades (and standard error) for neutrophil infiltration and edema in the muscular and serosal layers of the colon.

could be observed. Areas of blood, drops of a viscous fluid, and small fibrin clots were observed over the serosa and the mesocolon. By 90 min after decompression, the serosa was still congested, and the wall was thicker and more edematous in appearance. By 12 h after decompression, the color of the serosa was still abnormal, and there were more fibrin clots. On the cut surface, submucosal edema, principally at the mesenteric border, was observed in most of the distended segments. At the end of the experimental period, the sham-operated segments had a normal appearance, except for small fibrin clots and a few foci of hemorrhage adjacent to the biopsy sites.

There were no obvious histologic changes in the colonic mucosa and submucosa over time. The seromuscular layer showed mild to intense hemorrhage, intense neutrophil accumulation, and various amounts of fibrin (Figure 2). These lesions developed principally after decompression. In the sham-operated group, the same lesions were observed, but to a lesser extent.

There were no statistically significant differences among times in each group or between groups at each time in the scores for epithelial cell detachment, edema, hemorrhage, or neutrophil accumulation in the colonic mucosa and submucosa (data not shown). However, in the serosa, the distended segments showed significantly greater hemorrhage, fibrin deposition, and lymphatic vessel distension, predominantly after decompression, than the control segments; the only significant difference between the 2 groups of horses was in the amount of hemorrhage after 4 h of distension (Figure 3). There were also significant increases after decompression in edema and neutrophil accumulation in the serosa and muscularis in both groups of horses (Figure 4). The neutrophil counts in the serosa were significantly increased in both groups, but only at 90 min after decompression were the counts greater in the distension group than in the sham-operated group (Figure 5A).

Only after decompression in the distension group were the MPO activity and hemoglobin concentration in the seromuscular layer of the colon significantly increased (Figures 5B and 5C).

The mean grade of neutrophil accumulation in the lung samples was significantly greater for the distension group than for the sham-operated group. Similarly, the mean MPO activity was significantly greater in the lung samples from the distension group than in those from the sham-operated group (Figure 6).

Discussion

Macroscopic observations of hemorrhage and fibrin deposition in the colonic serosa of the distension group were confirmed by light microscopic analysis. Although most of the horses had seromucosal macroscopic edema at the end of the experiment, there were no significant increases in microscopic scores for this variable in the distended segments compared with the control segments. Mild submucosal edema in the control samples (possibly caused by manipulation) and the site of sample collection for microscopy may explain this finding: edema was evident macroscopically at the mesenteric border, but the samples were collected at the antimesenteric border, as a previous study had shown this to be the site most affected by ischemia caused by luminal distension (7). Collection of

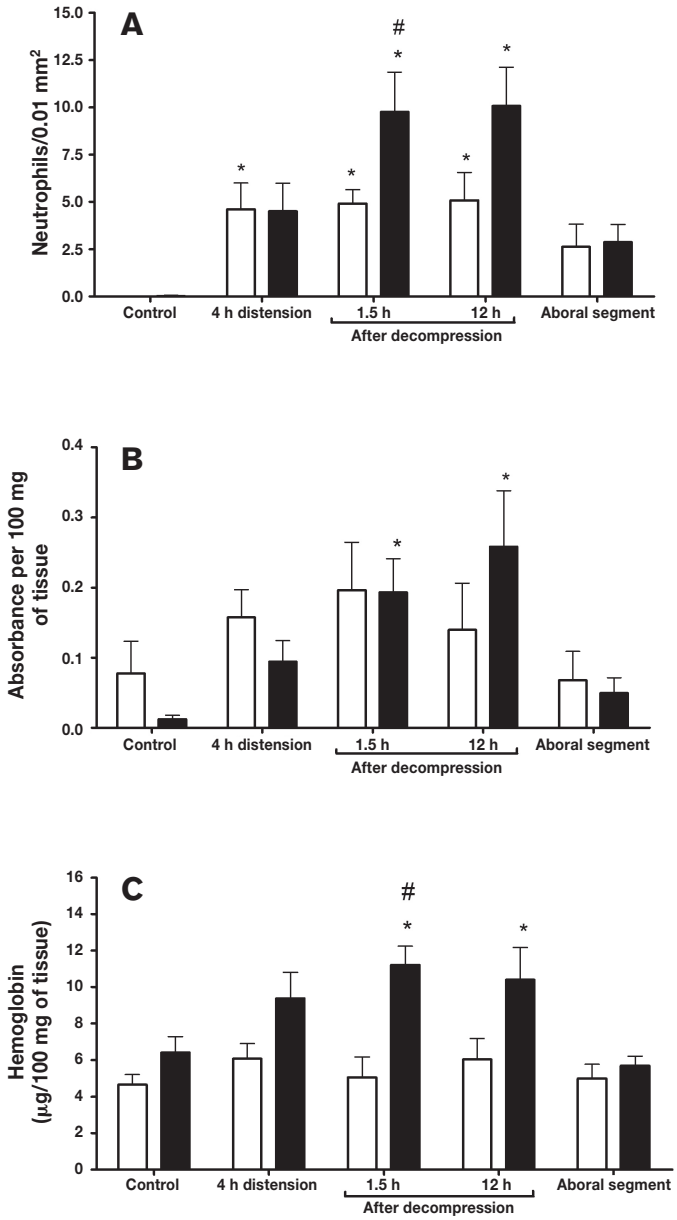

Figure 5. Means (and standard error) for neutrophil count in the serosa (A) and MPO activity (B) and hemoglobin concentration (C) in the seromuscular layer.

samples from around the entire perimeter of the small colon might have shown changes. Submucosal edema associated with increased microvascular permeability was observed in the jejunum after 1 h (13) and 2 h (14) of luminal distension.

The local lesions of the small colon in the distension group were observed almost exclusively in the seromuscular layer, corroborating previous findings with the same model that the muscularis and serosa are the intestinal layers most affected by the microvascular ischemia caused by luminal distension of the small colon (7). In the present study, it was not possible to identify relevant lesions in the mucosa even after 4 h of distension and 12 h of reperfusion; the same previous study also showed that the mucosa was not affected by the microvascular ischemia caused by such distension (7). Taken together, these findings provide new clues regarding the vascular alterations caused by luminal distension in the intestinal wall and indicate that the previous methodology (7), whereby microspheres were quantified histologically in the intestinal layers, could be useful for future studies of microvascular alterations in the equine small colon.

In agreement with the present study, several other models using luminal distension in equine jejunum demonstrated prevalent lesions in the seromuscular layer and only discrete lesions in the mucosa, indicating selective ischemia in the outer layer (6,8,12,15).

The local lesions were clearly worse in the distension group than in the sham-operated group. However, some of the lesions, mainly the edema and neutrophil accumulation in the seromuscular layer, were caused in part by surgical manipulation and biopsy, since they were also seen in the sham-operated group. Similar alterations have been described in intestinal control segments subjected to biopsy (16). Such lesions were not observed in samples obtained at the end of the experiment from the aboral segment of the small colon, which was not surgically manipulated. This finding highlights the importance of using a sham-operated group as a control in the scientific study of intestinal distension.

Neutrophil accumulation in the seromuscular layer occurred in both groups, but quantitative analysis demonstrated that the numbers in the distension group after 90 min of reperfusion were almost twice those in the sham-operated group ($P = 0.02$). A similar increase in the number of neutrophils in the serosa was detected in the jejunum after 2 h of distension: 39.5 cells/0.01 mm^2 versus 9.3 cells/0.01 mm^2 in the distension and sham-operated groups (14).

In the distension group, but not in the sham-operated group, significant increases, compared with predistension values, were observed in MPO activity and hemoglobin concentration in the seromuscular layer. Scores for hemorrhage, fibrin deposition, and lymphatic vessel dilation in the serosa were also significantly greater in the distension group. These increases occurred predominantly after decompression, suggesting ischemia–reperfusion injury as a factor in the development of the lesions. The increases in hemoglobin concentration and in hemorrhage and fibrin deposition are consistent with the macroscopic findings and indicate that the distended small colon may be predisposed to adhesions. In fact, several experiments have suggested that ischemia seems to be fundamental to the formation of postoperative adhesions (17).

There was some discrepancy between MPO activity and neutrophil count in the seromuscular layer. Whereas the number of neutrophils increased in both groups during reperfusion, MPO activity increased at this time only in the distension group. Myeloperoxidase activity has frequently been used as indicative of the amount of neutrophil infiltration in the intestine and lungs of laboratory animals (12) and horses (18–21). However, a study comparing several forms of quantification of inflammatory cells in the mucosa of the equine large colon concluded that MPO activity does not correlate well with neutrophil score or count (18).

Differences in analytic method, high variability among measurements, and the production of MPO by other cells in the tissue, such as eosinophils and macrophages, have been suggested as explaining such discrepancies (18,20). In the present study, the seromuscular layer was isolated from the remainder of the intestinal wall by dissection

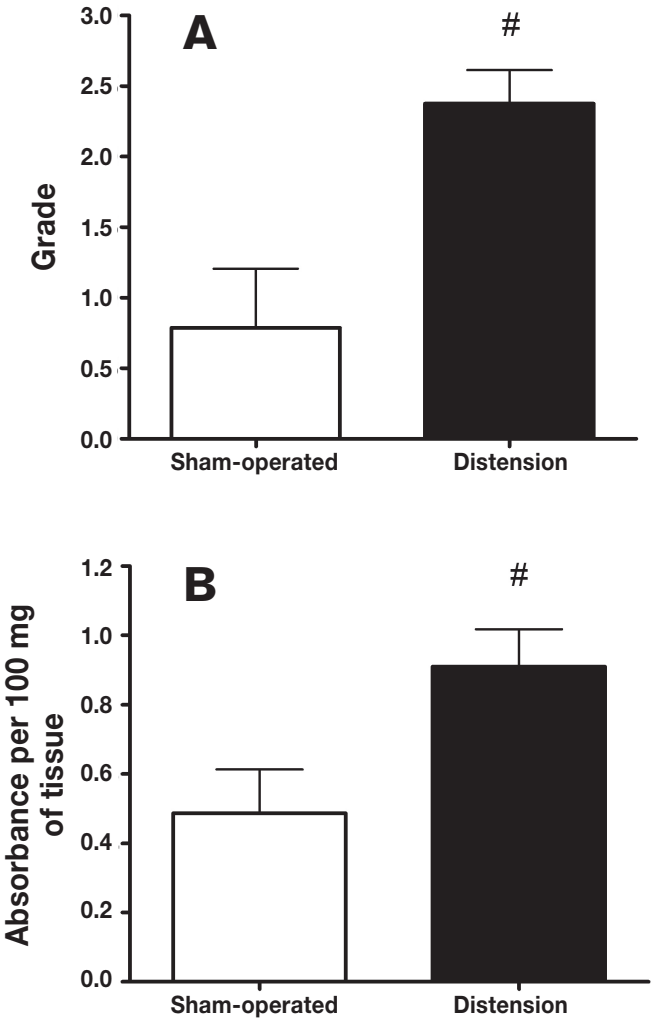

Figure 6. Mean grades (and standard error) for neutrophil accumulation (A) and MPO activity (B) in lung samples from the distension (*n* = 4) and sham-operated (*n* = 7) groups of horses.

to avoid the influence of cells commonly found in the mucosa and submucosa. Therefore, the increase in MPO activity observed only in the distension group may be related to the abundance of neutrophils. It could also be explained by neutrophil activation during reperfusion. Studies in vitro have demonstrated a high correlation between MPO activity and the number of activated neutrophils in horses (21). Thus, the increase in MPO verified at 1.5 and 12 h after the end of distension could be due to the ischemia–reperfusion process in the seromuscular layer. Corroborating evidence was provided by a study showing that horses with intestinal obstruction had high plasma MPO activity (22).

The greatest neutrophil activation in the distension group, with the consequent increase in liberation of MPO, could also explain the blood in the seromuscular layer. Benbarek et al (21), studying the effect of the supernatant of activated neutrophils on cultivated equine endothelial cells, verified crescent-shaped endothelial cells that were positively correlated with MPO activity in the supernatant. Myeloperoxidase can damage endothelial cells by converting the hydrogen peroxide that originates from neutrophils into hypochlorous acid, another potent oxidant (3).

In laboratory animals, inhibition of neutrophil accumulation or activation can ameliorate ischemia–reperfusion lesions (12). However, this was not demonstrated when a cellular filter was used to remove neutrophils from the jejunal blood flow in an ischemia–reperfusion model using an extracorporeal circuit (23). Although differences exist among species and ischemic models, the limitation of in vitro studies must also be considered to explain this discrepancy.

Most of the pathological changes in the seromuscular layer of the distension group were significant in relation to baseline values not at the end of distension but predominantly at least 90 min after decompression. This finding indicates progression of the lesions after the restoration of microvascular blood flow at the end of luminal distension. Progression of the lesions after 90 min of reperfusion has also been shown after 90 and 180 min of total ischemia of the small colon (11). Together, these findings suggest a reperfusion lesion in the equine small colon.

Controversy exists regarding the occurrence of reperfusion injury in the equine gastrointestinal tract. In the jejunum (1) and large colon (2), lesions caused by ischemia followed by reperfusion were greater than those observed after the same period of uninterrupted ischemia, which unquestionably demonstrates the occurrence and relevance of reperfusion. However, other models have failed to demonstrate the progression of lesions after ischemia in the jejunum (19) and large colon (24,25). In addition, several types of therapy directed at inhibiting the formation of oxygen free radicals, a major pathophysiologic mediator of reperfusion injury, failed to protect jejunum (1,19) and large colon (5,26) subjected to ischemia and reperfusion. Thus, the clinical relevance of reperfusion injury in equine intestine has been questioned (27). However, more recently, anti-inflammatory (28) and multimodal (29–32) therapies have demonstrated some protection of jejunum subjected to ischemia and reperfusion and have reignited clinical and scientific interest in reperfusion injury.

One of the main characteristics of the reperfusion injury process in the gastrointestinal tract is its capability to induce lesions in distant organs (3). The greater neutrophil accumulation and MPO activity in the lungs of the distension group indicates that a typical process of ischemia–reperfusion injury taking place in the seromuscular layer of the small colon was sufficient to induce remote lesions in our model. This finding could be relevant to our pathophysiological understanding of the ischemic lesions in the equine gastrointestinal tract. In laboratory animals, several studies using cranial mesenteric artery occlusion have demonstrated that remote lesions are associated with the systemic inflammatory response syndrome (SIRS), multiple organ dysfunction syndrome (MODS), and high mortality rates (12,33). Involved in this process are endotoxin (34) and several endogenous inflammatory mediators, such as leukotriene B4, platelet-activating factor, phosphodiesterases, bradykinin, and cytokines, mainly tumor necrosis factor alpha (34). Neutrophil recruitment has been shown to be a determinant in this process (33,35).

Other evidence of remote lesions in horses has been reported. Grulke et al (36) found pancreatic lesions and increased levels of plasmatic trypsin in horses with intestinal strangulating obstructions. Recently, Black et al (37) reported leukocyte accumulation in lamellar tissue during early stages of laminitis, probably the most common and evident clinical form of remote lesions in horses secondary to ischemic and inflammatory conditions of the gastrointestinal tract.

It is premature to consider the clinical significance of neutrophil accumulation in the lungs of horses with intestinal ischemia and reperfusion, as verified in the present study. It seems that equine neutrophils have a self-limited proteinase system, which may explain high neutrophil numbers in airways without extensive destruction of the lung parenchyma in cases of heaves (38). In fact, pulmonary disease in not a common complication after colic; however, future research may clarify whether neutrophil accumulation can be associated with other complications, such as anesthesia-related death, which has a high rate in horses with colic (39).

In summary, although luminal distension of the equine small colon was not sufficient to cause mucosal damage, it was sufficient to incite local lesions in the seromuscular layer of the colon and remote lesions in the lungs, both of which are typical with ischemia and reperfusion. These findings provide supportive evidence for the occurrence of reperfusion injury and SIRS in horses with intestinal obstruction and justify further investigation into the occurrence of these lesions and, in particular, methods for preventing or limiting their extent.

Acknowledgments

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References

1. Horne MM, Pascoe PJ, Ducharme NG, Barker IK, Grovum WL. Attempts to modify reperfusion injury of equine jejunal mucosa using dimethylsulfoxide, allopurinol, and intraluminal oxygen. Vet Surg 1994;4:241–249.
2. Moore RM, Bertone AL, Muir WW, Stromberg PC, Beard WL. Histologic evidence of reperfusion injury in the large colon of horses after low-flow ischemia. Am J Vet Res 1994;55:1434–1443.
3. Moore RM, Muir WW, Granger DN. Mechanisms of gastrointestinal ischemia-reperfusion injury and therapeutic interventions: a review and its implications in the horse. J Vet Intern Med 1995;9:115–132.
4. Wilson DV, Patterson JS, Stick JA, Provost PJ. Histologic and ultrastructural changes after large-colon torsion, with and without use of a specific platelet-activating factor antagonist (WEB 2086) in ponies. Am J Vet Res 1994;55:681–688.
5. Moore RM, Muir WW, Bertone AL, Beard WL, Stromberg PC. Effects of dimethyl sulfoxide, allopurinol, 21-aminosteroid U-74389G and manganese chloride on low-flow ischemia and reperfusion of the large colon in horses. Am J Vet Res 1995;56:671–687.
6. Dabareiner RM, Sullins KE, White NA, Snyder JR. Serosal injury in the equine jejunum and ascending colon after ischemia-reperfusion or intraluminal distention and decompression. Vet Surg 2001;30:114–125.
7. Faleiros RR, Macoris DG, Alessi AC, Saquetti CH, Rasera L. Effect of intraluminal distention on microvascular perfusion in the equine small colon. Am J Vet Res 2002;63:1292–1297.
8. Lundin C, Sullins KE, White NA, Clem MF, Debowes RM, Pfeiffer CA. Induction of peritoneal adhesions with small intestinal ischaemia and distention in the foal. Equine Vet J 1989; 21:451–458.
9. Malone EC, Kannan MS. Effects of intestinal ischemia on in vitro activity of adjacent jejunum in samples obtained from ponies. Am J Vet Res 2001;62:1973–1977.
10. Faleiros RR. Obstrução experimental do cólon menor eqüino: aspectos clínicos, patológicos e terapêuticos [PhD dissertation]. Jaboticabal, São Paulo, Brazil: Universidade Estadual Paulista, 2003:29–57.
11. Faleiros RR, Alves GES, Santos RL, Marques AP Jr, Macoris DG. Experimental ischemia and reperfusion in equine small colon. Arq Bras Med Vet Zootec 2001;53:341–350.
12. Souza DG, Coutinho SF, Silveira MR, Cara DC, Teixeira MM. Effects of a BLT receptor antagonist on local and remote reperfusion injuries after transient ischemia of the superior mesenteric artery in rats. Eur J Pharmacol 2000;403:121–128.
13. Nieto JE, Van Hoogmoed LM, Spier SJ, Vatistas NJ, Snyder JR, Timmerman BL. Use of an extracorporeal circuit to evaluate effects of intraluminal distention and decompression on the equine jejunum. Am J Vet Res 2002;63:267–275.
14. Dabareiner RM, White NA, Donaldson LL. Effects of intraluminal distention and decompression on microvascular permeability and hemodynamics of equine jejunum. Am J Vet Res 2001;62: 225–236.
15. Allen D, White NA, Tyler DE. Morphologic effects of experimental distention of equine small intestine. Vet Surg 1988;17:10–14.
16. Faleiros RR. Isquemia e reperfusão experimental no cólon menor de eqüinos [MSc thesis]. Belo Horizonte, Minas Gerais: Escola de Veterinária, Universidade Federal de Minas Gerais, 1997:1–50.
17. Southwood LL, Baxter GM. Current concepts in management of abdominal adhesions. Vet Clin North Am Equine Pract 1997;13: 415–434.
18. Moore RM, Bertone AL, Bailey MQ, Muir WW, Beard WL. Neutrophil accumulation in the large colon of horses during low-flow ischemia and reperfusion. Am J Vet Res 1994;55: 1454–1463.
19. Vatistas NJ, Snyder JR, Hildebrand SV, et al. Effects of U-74389G, a novel 21-aminosteroid, on small intestinal ischemia and reperfusion injury in horses. Am J Vet Res 1996;57:762–770.
20. McConnico RS, Weinstock D, Poston ME, Roberts MC. Myeloperoxidase activity of the large intestine in an equine model of acute colitis. Am J Vet Res 1999;60:807–813.
21. Benbarek H, Grulke S, Deby-Dupont G, et al. Cytotoxicity of stimulated equine neutrophils on equine endothelial cells in culture. Equine Vet J 2000;32:327–333.
22. Deby-Dupont G, Grulke S, Caudron I, et al. Equine neutrophil myeloperoxidase in plasma: design of a radio-immunoassay and first results in septic pathologies. Vet Immunol Immunopathol 1998;66:257–271.
23. Van Hoogmoed LM, Snyder JR, Nieto JG, Harmon FA, Timmerman BL. Effect of a leukocyte-depleting filter in an

extracorporeal circuit used for low-flow ischemia and reperfusion of equine jejunum. Am J Vet Res 2001;62:87–96.
24. Reeves MJ, Vansteenhouse J, Stashak TS, Yovich JV, Cockerell G. Failure to demonstrate injury following ischaemia of the equine large colon using dimethyl sulphoxide. Equine Vet J 1990;22: 126–132.
25. Wilkins PA, Ducharme NG, Lowe JE, Schwark WS, Meschter C, Erb HN. Measurements of blood flow and xanthine oxidase activity during postischemic reperfusion of large colon of ponies. Am J Vet Res 1994;55:1168–1177.
26. Henninger DD, Snyder JR, Pascoe JR, Dilling GW. Microvascular permeability changes in ischemia/reperfusion injury in the ascending colon of horses. J Am Vet Med Assoc 1992;201: 1191–1196.
27. Blikslager AT, Roberts MC, Gerard MP, Argenzio RA. How important is intestinal reperfusion injury in horses? J Am Vet Med Asooc 1997;211:1387–1389.
28. Alves GES, Matos JJRT, Faleiros RR, Santos RL, Marques AP Jr. Efeito da hidrocortisona sobre a lesão de reperfusão e reparação da mucosa após isquemia venosa experimental no jejuno de eqüinos. Arq Bras Med Vet Zootec 2003;55:539–549.
29. Dabareiner RM, White NA, Snyder JR, Feldman BF, Donaldson LL. Effects of Carolina rinse solution, dimethyl sulfoxide, and the 21-aminosteroid, U-74389G, on microvascular permeability and morphology of the equine jejunum after low-flow ischemia and reperfusion. Am J Vet Res 2005;66:525–536.
30. Dabareiner RM, White NA 2nd, Donaldson L. Evaluation of Carolina rinse solution as a treatment for ischaemia reperfusion of the equine jejunum. Equine Vet J 2003;35:642–646.
31. Van Hoogmoed LM, Snyder JR, Nieto J, Harmon FA. In vitro evaluation of an intraluminal solution to attenuate effects of ischemia and reperfusion in the small intestine of horses. Am J Vet Res 2002;63:1389–1394.
32. Young BL, White NA, Donaldson LL, Dabareiner RM. Treatment of ischaemic jejunum with topical and intraluminal Carolina rinse. Equine Vet J 2002;34:469–474.
33. Souza DG, Teixiera MM. The balance between the production of tumor necrosis factor-alpha and interleukin-10 determines tissue injury and lethality during intestinal ischemia and reperfusion. Mem Inst Oswaldo Cruz 2005;100(Suppl 1):59–66.
34. Wagner JG, Roth RA. Neutrophil migration during endotoxemia. J Leukoc Biol 1999;66:10–24.
35. Xiao F, Eppihimer MJ, Young JA, Nguyen K, Carden DL. Lung neutrophil retention and injury after intestinal ischemia/ reperfusion. Microcirculation 1997;4:359–367.
36. Grulke S, Deby-Dupont G, Cassart D, et al. Pancreatic injury in equine acute abdomen evaluated by plasma trypsin activity and histopathology of pancreatic tissue. Vet Pathol 2003;40:8–13.
37. Black SJ, Lunn DP, Yin C, Hwang M, Lenz SD, Belknap JK. Leukocyte emigration in the early stages of laminitis. Vet Immunol Immunopathol 2006;109:161–166.
38. Jefcoat AM, Wagner JG, Robinson NE. The neutrophil: understanding ancient and powerful responses in the inflammatory balance. Equine Vet J 2003;35:5–6.
39. Johnston GM, Eastment JK, Wood JLN, Taylor PM. The confidential enquiry into perioperative equine fatalities (CEPEF): mortality results of phases 1 and 2. Vet Anaesth Analg 2002;29: 159–170.